

## CLAIMS

1. A method of detecting a fluorescent molecule in a test sample, comprising the following steps:
  - 5 (a) a step of measuring in a time-dependent manner individual fluorescence intensities of a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime; and
  - (b) a step of comparing the measured fluorescence intensities.
- 10 2. A method of detecting a substance to be measured in a test sample, comprising the following steps:
  - (a) a step of labeling the substance to be measured with a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime;
  - (b) a step of measuring in a time-dependent manner individual fluorescence intensities of
  - 15 the fluorescent molecules labeling the substance; and
  - (c) a step of comparing the measured fluorescence intensities.
- 20 3. A method of judging the type of a substance to be measured in a test sample, comprising the following steps:
  - (a) a step of labeling the substance to be measured with a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime;
  - (b) a step of measuring in a time-dependent manner individual fluorescence intensities of the fluorescent molecules labeling the substance;
  - (c) a step of comparing the measured fluorescence intensities; and
  - 25 (d) a step of judging the types of the substances to be measured using the intensity ratios obtained by the comparison.
- 30 4. The method according to any one of claims 1 to 3, wherein the plurality of species of fluorescent molecules comprise fluorescent molecules belonging to individual groups of different three or more groups selected from the group consisting of a group having an inherent fluorescence lifetime of 0.01 ns or more and less than 1.0 ns; a group having an inherent fluorescence lifetime of 1.0 ns or more and less than 2.0 ns; a group having an inherent fluorescence lifetime of 2.0 ns or more and less than 3.0 ns; a group having an inherent fluorescence lifetime of 3.0 ns or more and less than 4.0 ns; a group
- 35 having an inherent fluorescence lifetime of 4.0 ns or more and less than 5.0 ns; a group

having an inherent fluorescence lifetime of 5.0 ns or more and less than 6.0 ns; and a group having an inherent fluorescence lifetime of 6.0 ns or more and less than 7.0 ns.

5            5. The method according to any one of claims 1 to 3, wherein the plurality of species of fluorescent molecules comprise three or more fluorescent molecules which are different from each other by 1.0 ns or more in fluorescence lifetime.

10           6. The method according to any one of claims 1 to 3, wherein the plurality of species of fluorescent molecules comprise three or more fluorescent molecules which are different from each other by a factor of 1.1 or more in fluorescence lifetime.

7. The method according to any one of claims 1 to 3, wherein the fluorescence lifetime is 30 ns or less.

15           8. The method according to claim 1, wherein at least one of the fluorescent molecules has a known concentration.

20           9. The method according to any one of claim 2 to 7, wherein at least one of the substances to be measured has a known concentration.

10. The method according to any one of claims 2 to 9, wherein the substance to be measured is a probe or target.

25           11. The method according to claim 10, wherein the probe or target is nucleic acid.

12. A method of analyzing a fluorescent molecule in a test sample, comprising the following steps:

30           (a) a step of measuring in a time-dependent manner individual fluorescence intensities of a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime and preparing a fluorescence lifetime function represented by the following formula I:

$$f(t) = \sum_{i=1}^k A_i \exp(-t / \tau_i) \quad (I)$$

where  $A_i$  is a coefficient;  $t$  is time; and  $\tau_i$  is fluorescence lifetime;  
and

- (b) a step of calculating fluorescence intensities using said function.

5            13. A method of analyzing a substance to be measured, comprising the following steps:

- (a) a step of labeling the substance to be measured with a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime;  
(b) a step of measuring in a time-dependent manner fluorescence intensities of the  
10 fluorescent molecules labeling the substance and preparing a fluorescence lifetime function represented by the following formula I:

$$f(t) = \sum_{i=1}^k A_i \exp(-t / \tau_i) \quad (I)$$

where  $A_i$  is a coefficient;  $t$  is time; and  $\tau_i$  is fluorescence lifetime;  
and

- 15 (c) a step of calculating fluorescence intensities using said function.

14. The method according to claim 12 or 13, wherein the calculation of fluorescence intensities is calculation of the product of coefficient  $A_i$  and fluorescence lifetime  $\tau_i$ .

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15. A method of judging the type of a gene, comprising the following steps:

- (a) a step of labeling the gene in a test sample with a plurality of species of fluorescent molecules each having an inherent fluorescence lifetime;  
(b) a step of measuring in a time-dependent manner fluorescence intensities of the  
25 fluorescent molecules labeling the substance and preparing a fluorescence lifetime function represented by the following formula I:

$$f(t) = \sum_{i=1}^k A_i \exp(-t / \tau_i) \quad (I)$$

where  $A_i$  is a coefficient;  $t$  is time; and  $\tau_i$  is fluorescence lifetime;

- (c) a step of calculating fluorescence intensities using said function to thereby detect the

fluorescence intensities of said fluorescent molecules; and

(d) a step of judging the type of the gene using said fluorescence intensities as indicators.

16. The method according to claim 15, wherein the calculation of fluorescence  
5 intensities is calculation of the product of coefficient  $A_i$  and fluorescence lifetime  $\tau_i$ .

17. The method according to any one of claims 1 to 16, wherein the fluorescence  
lifetime of at least one of the fluorescent molecules is known.

10 18. A reagent or kit for detecting a substance to be measured, comprising a  
plurality of species of fluorescent molecules each having an inherent fluorescence lifetime.

19. An apparatus for detecting a fluorescent molecule in a test sample, comprising  
the following means:

- 15 (a) means for measuring in a time-dependent manner individual fluorescence intensities of  
a plurality of species of fluorescent molecules each having an inherent fluorescence  
lifetime; and  
(b) means for comparing the measured fluorescence intensities.

20 20. An apparatus for quantitatively determining a substance to be measured in a  
test sample, comprising the following means:

- (a) means for labeling the substance to be measured with a plurality of species of  
fluorescent molecules each having an inherent fluorescence lifetime;  
(b) means for measuring in a time-dependent manner individual fluorescence intensities of  
25 the fluorescent molecules labeling the substance; and  
(c) means for comparing the measured fluorescence intensities.